74-Plat
Measuring DNA Bending and Twisting Flexibility in E. Coli
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DNA is a curious molecule. In vitro, DNA has been shown to be a rigid polymer resistant to bending and twisting over distances of <200 bp. In vivo, DNA demonstrates the ability to form loops over similar and even smaller distances. Cellular DNA looping plays an essential role in chromatin condensation, recombination, and transcriptional regulation. Architectural proteins, in both eukaryotic and prokaryotic organisms, bind non-specifically to DNA, perhaps enhancing apparent DNA flexibility by inducing transient bends and kinks. Our lab is interested in studying the interplay of intrinsic DNA stiffness and architectural proteins in modulating DNA flexibility in vivo. We utilize a classical in vivo bacterial looping system, based on the lactose operon, to ask these questions.
We set out to test the hypothesis that DNA looping in this system simply increases repressor concentration at the proximal operator that represses the test promoter. This hypothesis predicts that loop size, not relative position of the auxiliary operator, should determine promoter repression. Two sets of looping constructs have been created to test this hypothesis. One set follows the classic looping model with a bacterial promoter located between the two operators. The behavior of these ''upstream'' looping constructs reflect phasing- and distance-dependent repression indicative of stable DNA loops significantly smaller than in the natural lactose operon. A second set of looping constructs places the auxiliary operator further downstream of the transcriptional start site. These ''downstream'' constructs allow us to assess repression when the promoter is not constrained to be part of the DNA loop. Loop-dependent transcriptional repression is also observed for ''downstream'' looping but, interestingly, repression is much weaker. These results suggest that the mechanism of promoter repression by DNA looping is more complex than simply increasing repressor concentration at the proximal operator.

75-Plat
Quadruplex-Based Technology for Nucleic Acid Amplification and Detection
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Polymerase chain reaction (PCR) has impacted nearly every field in molecular biology, genetics and forensic science. Numerous applications of PCR have been described for basic scientific purposes, as well as for diagnosis of hereditary and infectious diseases. This is particularly true for real-time PCR (RT-PCR), which detects and quantifies product molecules within PCR reaction vessels. However, PCR is still considered a daunting task due to many variables in the reaction, temperature cycling and complicated quantification methods. Current RT-PCR specific probes (Molecular Beacons, TaqMan, Scorpions) require costly synthesis and considerable effort to achieve optimal sensitivity. Typically, a fluorophore-quencher pair is attached to the ends of a probe, which doesn't fluoresce when free in solution. Upon probe hybridization to an amplicon, the fluorophore is separated from the quencher and a signal is released.
Recently we have discovered that the free energy of DNA quadruplexes can be used to drive unfavorable (endergonic) reactions of nucleic acids (e.g., isothermal PCR). The key point of quadruplex-driven reactions is that some G-rich sequences are capable of forming quadruplexes with significantly more favorable thermodynamics than the corresponding DNA duplexes. The energy of quadruplex formation can be used to drive PCR at constant temperature or DNA signal amplification. In addition, fluorescent nucleotide analogs incorporated and fully quenched within the primers regain maximum emission upon quadruplex formation, allowing very simple and accurate detection of product DNA. Thermodynamic and fluorescent bases of quadruplex priming amplification will be discussed.
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76-Plat
The Cost of Being Right During Replication
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DNA replication needs to be both faithful and processive. DNA polymerases replicate DNA, using a template strand to add the complementary nucleotide to the synthesizing primer (polymerization). Family A DNA polymerases, like T7 DNA polymerase (DNAp), boost their fidelity by proofreading the primer. In the event of a mismatch, the erroneous nucleotide is excised at the exonuclease active site (proofreading). Proper balance between polymerization and proofreading is essential to achieve faithful but productive replication.
Here we report on a single-molecule study of DNAp using optical tweezers. In our setup we can directly measure the individual rates of replicating, proofreading and pausing single DNA polymerases at various tensions. With a spatial and temporal resolution of 25 bp and 0.2 sec, we can study DNAp in far more detail than previous single-molecule studies.
We find that the rate of polymerization decreases with tension on the DNA but, notably, doesn't stall at high tension. Next, the rate of processive proofreading is constant over all measured tensions which, surprisingly, include tensions of only a few pN. This means that the proofreading state of DNAp is highly accessible, independent of perturbations of the primer-template structure, and that during proofreading tens of nucleotides at a time are removed.
Our observations indicate that the current kinetic model for DNAp is not sufficient. The length of the pauses in between events are distributed as a double exponential, arguing against a single inactive state. Therefore, we introduce an updated model that contains an extra state that might play a role in balancing polymerization and proofreading. The conclusion seems that the cost of being right (i.e. error-free replication) outweighs the price a cell pays (i.e. the regular removal of correct base pairs) for picking up errors during replication.

77-Plat
The Dynamic Interplay Between Telomere-Binding Proteins POT1 and TPP1 in G-Quadruplex DNA
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Telomere-binding proteins, like POT1 (protection of telomeres protein 1) and TPP1, are important for the maintenance of telomere integrity and are known to regulate telomerase to ensure that an appropriate length of structural DNA is maintained. Specifically in humans, hPOT1 is thought to suppress unwanted DNA repair by sequestering the single-stranded telomere overhang. Previous studies reported that POT1 binds to telomeric DNA in a sequence specific manner whereas TPP1 has no interaction with DNA on its own. POT1 and TPP1 as a complex have been shown to enhance telomerase recruitment and processivity. Despite the wealth of biochemical and structural data, the mechanistic basis of these activities remain enigmatic.
Using single molecule FRET (Förster Resonance Energy Transfer) and PIFE (Protein Induced Fluorescence Enhancement) techniques on various lengths of telomeric DNA consisting of TTAGGG repeats, we observed that POT1 binds to the pre-folded G-quadruplex in a step-wise manner from 3' to 5'. Consistent with previous findings, POT1 binds stably to the telomeric DNA, and thereby sequesters it. In contrast, POT1 and TPP1 exhibit dynamic behavior i.e sliding back and forth on telomeric DNA. This may explain how POT1-TPP1 contributes to the recruitment and processivity of telomerase by making the telomeric DNA accessible and providing mobility for telomerase. In addition, we observe that TPP1 alone displays transient interaction with single stranded DNA in a non-sequence specific manner. Our result uncovers the molecular mechanism and provides the dynamic axis to telomere regulation.

78-Plat
Micromechanics of Human Mitotic Chromosomes
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Eukaryote cells completely reorganize their long chromosomal DNAs to folded mitotic chromosomes to facilitate their physical segregation during mitosis. The internal organization of mitotic chromosomes remains unclear. We report biophysical experiments on single mitotic chromosomes from human cells, where isolated single human chromosomes were studied by micromanipulation and nanonewton-scale force measurement to understand chromosome connectivity and topology. We demonstrated that 4 base blunt-cutting restriction enzymes completely dissolved single metaphase human chromosomes, while proteases did not cut through human chromosomes, and led to reduction of their elasticity. Our results rule out the possibility that the mitotic chromosome is structured on a mechanically contiguous internal protein scaffold. Instead, mitotic chromosomes have a ''chromatin network'' organization, where chromatin fiber inside each chromosome is tethered to itself, by crosslinking proteins.
We further looked into the effect of RNAi knockdowns of a major chromosome-organizing protein– condensin on mototic chromosome organization. We found the stiffness of human chromosomes goes down by almost 10 fold in condensin depleted cells, compared